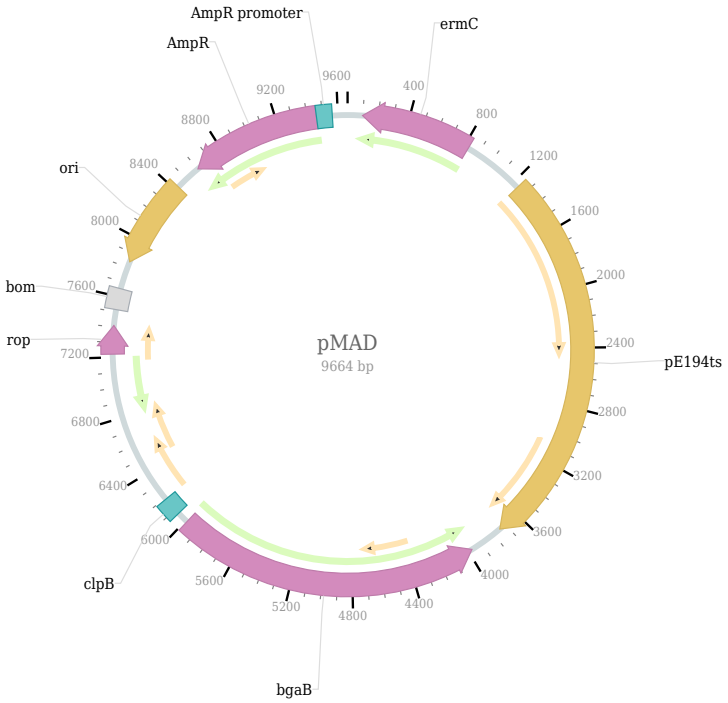
pMAD

载体编号 Vector ID	V007385
抗性 Antibiotic Resistance	Ampicillin
长度 Length	9664 bp
载体类型 Vector Type	Prokaryotic Expression Vectors

产品信息

复制子 Replication Origin	ori
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
备注 Comment	The pMAD vector is a shuttle vector designed for efficient allelic replacement in naturally nontransformable, low-GC-content Gram-positive bacteria. It features two key components: (i) a temperature-sensitive replication origin (pE194ts) derived from the Staphylococcus aureus plasmid pE194, which enables replication at permissive temperatures (≤32°C) but is fully blocked at nonpermissive temperatures (≥37°C); and (ii) a constitutive clpB promoter (pclpB) from Staphylococcus aureus that drives expression of the thermostable β-galactosidase gene (bgaB) from Bacillus stearothermophilus, facilitating blue-white screening on X-Gal plates without IPTG induction. Below is the step-by-step protocol for gene deletion using this vector. 1. Recombinant vector construction: Tandemly assemble "upstream homology arm of target gene – selection cassette (in the middle) – downstream homology arm", and clone into pMAD's multiple cloning site (e.g., BamHI/NcoI). 2. Transformation & primary screening: Electrotransform recombinant plasmid into Gram-positive bacteria; spread on "erythromycin + X-Gal" solid medium, incubate at 30°C for 2–3 days. Blue colonies indicate successful vector entry and free replication (pE194ts functions at permissive temperature; bgaB expressed via pclpB promoter). 3. Single crossover (vector integration): Pick 3–5 blue colonies, inoculate into erythromycin-containing liquid medium, shake-culture at 39–42°C for 6–12h (nonpermissive temperature blocks pE194ts replication, forcing single crossover-mediated chromosomal integration). Serial dilute and spread on "erythromycin + X-Gal" medium, incubate at 39–42°C for 18–24h. Select uniform blue colonies (successful integrants: erythromycin-resistant, vector retained). 4. Double crossover (gene deletion + vector excision): Pick 1–2 blue integrant colonies, inoculate into antibiotic-free liquid medium, culture at 30°C for 6h (permissive temperature promotes double crossover and vector excision from chromosome). Transfer to 42°C for 3–6h (nonpermissive temperature eliminates non-excised vectors). 5. Double crossover screening: Serial dilute and spread on "antibiotic-free + X-Gal" medium. White colonies = successful double crossover (vector lost, bgaB removed, target gene deleted); blue colonies = unexcised integrants (discard).

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 μl for 5 μg plasmid, and 100 μl for 100 μg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.